L. Jenni and R. F. Steiger. — Viruslike particles in the Tsetse fly, *Glossina morsitans* sspp. Preliminary results. (With 2 figures) [1]

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In the course of ultrastructural investigations on the development of *Trypanosoma brucei* [2] in *Glossina*, viruslike particles (VLP) were found in cytoplasmic vacuoles of the salivary gland (Jenni 1973) and midgut epithelium.

Haematophagous insects are known to be biological transmitters of arboviruses (Casals and Reeves 1965). So far, four groups of arthropods are known to play important vector roles in this context (Chamberlain 1968), namely mosquitoes, ticks, sandflies (Phebotomus), and bloodsucking gnats (Culicoides).

The growth of arboviruses in salivary gland epithelial cells of the vectors is a general feature (Mims *et al.* 1966; Chamberlain 1968; Janzen *et al.* 1970; Larsen and Ashley 1971; Whitfield *et al.* 1971), whereby mature virions are released into the saliva and transmitted by a subsequent bite to vertebrate hosts (La Motte 1960).

It is the aim of the present study to give an ultrastructural account of the VLP encountered in *G. morsitans*, and to correlate our results to fine structural data on the development of proven arboviruses mostly studied in cultured cell lines (reviewed by Joklik and Zweerink 1971; Grimley *et al.* 1972; Gil-Fernandez *et al.* 1973).

Material and Methods

Glossina morsitans centralis (Machado) were derived from pupae collected in Singida, Tanzania, and *G. m. orientalis* (Vanderplank) from pupae produced in a laboratory colony (Tsetse Res. Lab., Bristol). The newly emerged flies got their first bloodmeal on rats and were subsequently fed daily on a clean ox or clean mice. The adult flies were kept in dark climatized fly rooms.

From day 20 after emergence onward, flies of both sexes were dissected, and the salivary glands and midguts processed for electron microscopy according to routine methods. Double-stained (uranyl acetate/lead citrate) ultrathin sections were examined and recorded in Philips EM 300 and Zeiss EM 9 electron microscopes.

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[2] *T. brucei* is the pathogenic agent of Nagana in African cattle.

Results

Cytoplasmic membrane-bound vacuoles (CV) measuring up to 2.5 μm in diameter are regularly found within epithelial cells of the midpart of the salivary glands (fig. 1). These CV are confined to the Golgi/ER region (fig. 1).

Inside these vacuoles spherical viruslike particles (VLP) composed of an electron-dense core and a membranous envelope can be seen (fig. 2 + inset). The core measures 240-260 Å, the envelope 480-520 Å in diameter. The envelope has "unit" membrane character (fig. 2, inset) and is approximately 70 Å thick. An electron-translucent zone of 150-200 Å separates the core from the membrane. At times, the VLP seem to have a hexagonal outline. In addition, the CV contain randomly oriented tubular and rodlike structures (Tup to 0.7 μm in length, 200-250 Å across, fig. 2), as well as spherical vesicular structures, which appear "empty",i.e. without an electron-dense core (fig. 2). Also myelinlike membranous whorls can be seen (fig. 2). The VLP seem to be closely associated with membranous rods.

In older flies (about day 70) the cytoplasmic vacuoles appear somewhat larger, and vesicular contents predominate with membrane-bound VLP being less frequent.

Very recently, the same kind of CV and VLP have been found in midgut epithelial cells, too.

Discussion

Different types of cytoplasmic vacuoles seem to play an important role in the morphogenesis of Group A arboviruses (Erlandson *et al.* 1967; Grimley *et al.* 1968, 1972). Friedman *et al.* (1972) showed that the "type 1 cytopathic vacuoles" (CPV-1) are involved in viral replication. Precursor nucleoids seem to assemble on the vacuolar membrane; they acquire their envelope by budding through this membrane (Acheson and Tamm 1967; Grimley *et al.* 1968). The cytoplasmic vacuoles found in the present study resemble those described by the latter authors ("CVP-1 *c*") and Gil-Fernandez *et al.* (1973).

The occurrence of tubular and myelin-like material together with enveloped virus particles in the lumen of vacuoles has also been reported (lit. cit.). "Whorled membranes" within cytoplasmic inclusions associated with viruslike particles have been described in leukemic bone marrow (Sun *et al.* 1973). Intravacuolar tubular structures have been suggested to give rise to larger particles of the Semliki Forest virus (SFV) (Erlandson *et al.* 1967).

The concept that the cytoplasmic vacuoles of cells infected with Group A and B arboviruses are derived from the Golgi and ER complex, respectively (Grimley *et al.* 1972; Janzen *et al.* 1970), may also prove true for the vacuoles in the present investigation. As a matter of fact, *G. morsitans* vacuoles stain

positive for acid phosphatase, and tracer experiments with Dextrafer [1] injected into the haemocoel revealed the marker in the same structures. They could represent degenerative "CPV-1 *c*" (GRIMLEY *et al.* 1968) in a late stage of infection. Lysosome-like bodies which contain small VLP and myelin figures, have been interpreted as part of a cellular defence mechanism (STEIGER *et al.* 1969).

The recent finding of VLP in corresponding CV of midgut epithelial cells of *G. morsitans* may support the idea that the insect gut is another site of viral replication (CHAMBERLAIN 1968).

As regards size and shape, the VLP of the Tsetse fly resemble arboviruses of Group A (CHAIN *et al.* 1966; ERLANDSON *et al.* 1967; JANZEN *et al.* 1970).

To prove their viral nature different experiments will be undertaken, such as isolation and propagation in susceptible cell lines and/or hosts, EM negative staining and an eventual serological characterization.

ZUSAMMENFASSUNG

Runde virus-ähnliche Partikel wurden in cytoplasmatischen Vakuolen des Speicheldrüsen- und Mitteldarmepithels von *Glossina morsitans sspp.* gefunden. Sie besitzen eine Hüllmembran und einen elektronendichten „Kern". Ihr Totaldurchmesser beträgt ca. 480-520 Å.

Ihre Feinstruktur wurde mit der definierter Arboviren verglichen. Der gegenwärtige Stand der Untersuchungen lässt aber noch keine Charakterisierung der gefundenen Partikel zu.

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FIG. 1.

25 days after emergence. A membrane-bound vacuole (v) in an epithelial cell of the salivary gland. Lumen of the gland (lu), Golgi complexes (g), mitochondrion (m) and rough endoplasmic reticulum (er).
15000 ×

FIG. 2.

27 days after emergence. Details from a CV. Enveloped viruslike particle (→) associated with rodlike structures (rs). "Empty" vesicles (≻), myelinlike whorls (my).
138000 ×

Inset: Detailed picture of a VLP. Note the dense core and the membranous enveloppe.
332000 ×